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Anti-erosive potential of amine fluoride, cerium chloride and laser irradiation application on dentine

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Abstract: METHODS: Ninety-six dentine samples were prepared from human premolars and randomly assigned to eight groups (G1-G8). Samples were treated for 30s with the following solutions: placebo (G1/G2), amine fluoride (Elmex fluid; G3/G4), cerium chloride (G5/G6) and combined fluoride/cerium chloride application (G7/G8). Samples of groups G2, G4, G6 and G8 were additionally irradiated with a carbon dioxide laser through the solutions for 30s. Acid resistance was assessed in a six-time 5-min consecutive lactic acid (pH 3.0) erosion model and calcium release was determined by atomic absorption spectroscopy (AAS). Furthermore, six additional samples per group were prepared and subjected to EDS-analysis. RESULTS: In the non-irradiated groups, specimens of G1 (placebo) showed the highest calcium release when compared to the other treatments (G3, G5 and G7). The highest acid resistance was observed for G7. In G3, calcium release was lower than in G5, but higher than in G7. In general (except for the placebo groups), calcium release in the laser-irradiated groups was higher compared with the respective non-irradiated groups. EDS showed a replacement of calcium by cerium and of phosphor by fluoride. CONCLUSION: The highest anti-erosive potential was found after combined cerium chloride and amine fluoride application. Laser irradiation had not adjunctive effect.

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Anti-erosive potential of amine fluoride, cerium chloride and laser irradiation application on dentine

Running title: Potential erosive protection by $CeCl_2$/AmF

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Key words: dentine, fluoride, cerium chloride, laser, erosion

Abstract:

Methods: Ninety-six dentine samples were prepared from human premolars and randomly assigned to eight groups (G1 to G8). Samples were treated for 30 s with the following solutions: placebo (G1/G2), amine fluoride (Elmex fluid; G3/G4), cerium chloride (G5/G6) and combined fluoride/cerium chloride application (G7/G8). Samples of groups G2, G4, G6 and G8 were additionally irradiated with a carbon dioxide laser through the solutions for 30 s. Acid resistance was assessed in a six-time 5-minutes consecutive lactic acid (pH 3.0) erosion model and calcium release was determined by atomic absorption spectroscopy (AAS). Furthermore, six additional samples per group were prepared and subjected to EDS-analysis.

Results: In the non-irradiated groups, specimens of G1 (placebo) showed the highest calcium release when compared to the other treatments (G3, G5 and G7). The highest acid resistance was observed for G7. In G3, calcium release was lower than in G5, but higher than in G7. In general (except for the placebo groups), calcium release in the laser-irradiated groups was higher compared with the respective non-irradiated groups. EDS showed a replacement of calcium by cerium and of phosphor by fluoride.

Conclusion: The highest anti-erosive potential was found after combined cerium chloride and amine fluoride application. Laser irradiation had not adjunctive effect.

Introduction:

Dental erosion is defined as enamel or dentine tissue mineral loss due to persisting contact of the substrate with acids or chelators in the absence of bacteria.[1] As this loss is irreversible, the prevention of erosive tooth wear is important.

Already in 1977, Davis and Winter[2] reported that the use of fluoridated tooth pastes could reduce erosive tooth wear when used before an erosive challenge. Numerous other studies have been performed to examine the anti-erosive different formulations of fluorides.[3-6] Fluorides do not only have a protective effect against erosion but also, and maybe more importantly, against caries by demineralization inhibition and remineralisation promotion of dental hard tissues.[7] Thus, the use of fluorides has caused a reduction in the prevalence of dental hard tissue loss due to caries over the past decades.[8-11] But apart from these positive effects, fluorides in high concentration may also exhibit negative local and systemic side effects such as dental and skeletal fluorosis.[10,12,13]

As many of the preventive measures concerning erosion prevention depend on patients compliance, the chance that the measures fail to prevent erosive tooth wear is still high.[14] Therefore, other professional preventive measures, like in-office use of highly concentrated fluoride solutions or varnishes or lasers[14] have been proposed.[6,15] In general, there is still need for improvements of efficacy regarding this problem, especially when combining these strategies.

A chemical alternative and supplementation, which avoids the possible negative side effects of fluorides was proposed by Zhang et al. (1999), who tested the use of rare earth element solutions and combinations with sodium fluoride solutions for the prevention of carious-like lesion.[16] The toxicity of lanthanum and cerium is lower than that of fluoride, and rare earth elements show a lower tendency to accumulate in the liver, kidney and brain.[17] It was shown by Zhang et al.[16] that the protective effects of the different lanthanum and cerium solutions were at least comparable with those of fluoride solutions. In a recent study, the use of a cerium solution and a combination with a fluoride solution showed promising results concerning the reduction of acid susceptibility of dentine.[3]

With regard to physical interventions, different studies have demonstrated the potential of lasers to change the chemical composition and morphology of enamel resulting in an inhibition of demineralisation.[14,18-21] In contrast, studies using dentine[22,23] as substrate are scarce and the demineralisation conditions simulated were more caries-like than erosion-like.[24]

The aim of the present in-vitro-study was to evaluate the protective effect of a cerium solution and a combination of cerium/fluoride solutions on the prevention of mineral loss under erosive conditions on dentine. Furthermore, a possible additive effect of concomitant laser irradiation through the solutions was tested.

Taking in consideration that the uptake of fluoride is enhanced by laser irradiation through topical applied fluoride[25] and the promising results by Wegehaupt et al.[3] concerning the protective effect of cerium, the hypothesis of the present study was that the mineral loss due to erosion would be significantly reduced after application of cerium solutions and that an additional laser irradiation could improve dentine protection against demineralisation by enhancing the uptake of cerium into dentine.

Material and methods:

Sample preparation

Ninety-six dentine samples were prepared from human premolars and were assigned to eight experimental groups (n = 12, per group). The premolars were extracted for orthodontic reasons. Extracted teeth were collected as anonymous by-products of regular treatment. As such, the local medical ethical committee stated that the performed research was not conducted under the regulations of the Act on Medical Research Involving Human Subjects.

Teeth were sectioned at the cementum-enamel junction with a water-cooled diamond disc and the pulp tissue was removed from the roots with endodontic files. The roots surfaces were cleaned by scaling and flattened for 2 min with Sof-Lex™ polishing discs (3-9 μm grit, 3M Espe, 3M AG, Rüschlikon, Switzerland). To ensure that the root surfaces were free of cementum, they were checked under an anatomic microscope at a magnification of 40x. From each root, four dentine samples were harvested. Two cylinders were gained from the buccal and two from the palatal root surface, by drilling with a water-cooled diamond trephine mill (inner diameter 5 mm). The first cylinder was harvested 1 mm apical from the cemento-enamel junction, the second apical from the first one.[3] The dentine cylinders were embedded in acrylic resin and the samples were randomly allocated to eight experimental groups (G1-G8). Until use, they were stored under moist conditions (100% humidity) with a maximum storage time of 10 days.

Study design and treatment

The placebo solution (Placebo) for groups G1 and G2 was prepared by mixing 0.10 g sodium benzoate with 99.90 g distilled water. For G3 and G4, a commercially available amine (9250 ppm Olaflur and 750 ppm Dectaflur) fluoride solution (AmF) (Elmex fluid, GABA International AG, Therwil, Switzerland) was used (pH 3.9). The cerium chloride solution ($CeCl_3$) for G5 and G6 was composed of 10.00 g Cer(III)chlorid, 0.10 g sodium benzoate and 89.90 g distilled water (pH 4.94). The samples in G7 and G8 were treated with a 1:1 mixture of amine fluoride solution (Elmex fluid) and cerium chloride solution (AmF / $CeCl_3$).

In G1, G3, G5 and G7, the solutions were applied using applicator tips (DENTSPLY DeTrey GmbH, Konstanz, Germany) to the dentine samples for 30 s under constant motion. In G2, G4, G6 and G8, the samples were concomitantly irradiated (& Laser) through the solutions with a carbon dioxide (CO_2) laser (Spectra DENTA II, Lutronic, Goyang, South Korea) while the solutions are applied on the dentine surface with the following settings: wavelength 10.6 µm; pulsed; power 0.5 W; frequency 20 Hz; pulse duration 100 µs and beam diameter 1.1 mm.

After the 30 s laser application period, the samples were rinsed with distilled water to remove excess solution.

Evaluation of the acid resistance

The acid resistance of the dentine samples was evaluated by measuring the calcium released during an erosive attack (EA). For the erosive attack 120 µl of lactic acid (pH 3.0) was applied on the samples surface six times for 5 min each. Each 5 min the acid was removed and new acid was applied. The acid used in each 5 min attack was mixed with the same amount of water and strontium chloride (0.25%) and the amount of calcium was measured by atomic absorption spectroscopy (2380 Atomic Absorption Spectrophotometer, Perkin-Elmer, Schwerzenbach, Switzerland) at 422.7 nm.

EDS analysis and mapping

X-ray energy-dispersive spectroscopy (EDS) analysis of the dentine surface was performed (SUPRA 50 VP and Genesis, Carl Zeiss GmbH, Oberkochen, Germany) to evaluate possible reactions of the different solutions with the human dentine. For each group, additional six dentine samples were prepared and treated with the respective solutions. The samples were desiccated for 4 weeks in blue silica gel (Silica gel blue, Fluka Analytical, Buchs, Switzerland). On each sample, three areas of 200 x 200 µm were measured (15 kV, 100 s).[26] The weight percentage of calcium, cerium, phosphor and fluoride were analysed stoichiometrically. In groups 1, 2, 3 and 4 the weight percentages of cerium were under the detection limit (0.1 wt%) and were therefore not included in the statistical analysis.

To assess the potential reaction of the solutions with the dentine in deeper layers and the chemical depositions, five extra dentine samples for each group were prepared, cut axially before EDS mappings of these surfaces were performed. As the weight percentages of cerium in groups 1, 2, 3 and 4 were under the detection limit no data of EDS analysis and EDS mapping are presented. The weight percentages of fluoride in groups 1, 2, 5 and 6 were too low, so no EDS mapping concerning fluoride is presented.

Data presentation and statistical analysis

For data presentation of the calcium release and the EDS data analysis, the mean values and standard deviations of calcium in each 5 min acid fraction and of weight percentages of calcium, phosphor, fluoride and cerium were calculated. Data analysis was performed using ANOVA and Scheffe`s post hoc tests. Significance level was set at $p < 0.05$.

Results:

Calcium release into the acid

The amount of calcium released into the lactic acid during each of the six erosive attacks (EA 1–EA 6) and cumulatively for the different treatment groups (G1-G8) is given in Table 1.

Comparisons of the solutions:

In the groups with no laser irradiation (G1, G3, G5 and G7), the statistically significantly highest amount of calcium released in the acid during all erosive attacks and cumulative was observed for the samples treated with the placebo solution (G1) while the lowest amounts of calcium released were found in the group treated with fluoride/cerium chloride solution (G7).

In the groups with additional laser irradiation (G2, G4, G6 and G8), the statistically significantly highest amounts of calcium released, during all erosive attacks and cumulative, were observed for the samples treated with the placebo solution and the cerium chloride solution (G2 and G6). The fluoride/cerium chloride solution group (G8) exhibited for all erosive attacks (EA 1–EA 6) and cumulatively the lowest release of calcium.

Comparisons between groups with or without additional laser irradiation with same solution:

No significant difference was found in the amount of calcium released in the acid during all erosive attacks (EA 1 – EA 6) and cumulatively for the groups treated with placebo solution (G1 and G2). In the remaining groups, the calcium releases in the groups with additional laser irradiation (G4, G6 and G8) were significant higher compared with the respective groups without laser irradiation (G3, G5 and G7).

EDS analysis and mapping

Results of EDS analysis are given in Table 2.

Highest weight percentages of surface phosphor were observed for the samples treated with the placebo solution (G1), placebo solution with additional laser irradiation (G2) and cerium chloride solution with additional laser irradiation (G6) with significantly lower amounts in the remaining groups G3, G4, G5, G7 and G8 ($p < 0.05$, respectively).

Lowest weight percentages of surface fluoride was found in G1, G2, G5 and G6 (placebo solution, placebo solution with additional laser irradiation, cerium chloride solution and cerium chloride solution with additional laser irradiation) while in G3 (fluoride), G4 (fluoride with additional laser irradiation), G7 (fluoride/cerium chloride solutions) and G8 (fluoride/cerium chloride solution with additional laser irradiation) the weight percentages of surface fluoride were higher ($p < 0.05$, respectively).

In G3 and G4 (fluoride and fluoride with additional laser irradiation) calcium content was significantly higher than in all other groups. In G5, G7 and G8 (cerium chloride solution, fluoride/cerium chloride solutions and fluoride/cerium chloride solution with additional laser irradiation) a decrease of the surface calcium content was observed, while in the same groups, the content of surface cerium increases.

Results of EDS mapping are illustrated in Figure 1.

In G3, G4 and G7 (fluoride, fluoride with additive laser irradiation and fluoride/cerium chloride groups) a loss of phosphate in a depth ranging between 1 µm (G4) and 3 µm (G3 and G7) was observed, while in the same areas a distinct uptake of fluoride was found. In G8 (fluoride/cerium chloride with additive laser irradiation) the same effect was observed but less pronounced.

In G7 (fluoride/cerium chloride solution) and G8 (fluoride/cerium chloride solution with additive laser irradiation), a reduced content of calcium was observed with an increased content of cerium in the same region.

Discussion:

This study investigated the protective effect of cerium chloride, amine fluoride and combined application with or without additional laser irradiation on the erosion of dentine.

Human dentine from premolar was used, whereas numerous other studies assessing dentine erosion used bovine tooth samples.[27-29] Reasons for using bovine dentine as substrate for these studies might be that it is easier to obtain a sufficient number of sound bovine teeth instead of human teeth.[30] Furthermore, the bovine teeth, in contrast with human teeth, have no caries or fluoride application history that might influence the erosive tooth wear or the interaction of dentine with fluorides or other applied chemical substances. Although, there are some good reasons for using xenogenic material to substitute human dentine, main disadvantage is that erosive tooth wear of human dentine is higher that that of bovine dentine.[31] Therefore, human dentine was preferred in the present study. Due to the use of more than one sample from a single tooth and the random allocation of the samples to the eight groups, a mixture of independent (samples from different teeth) and dependent (samples from the same tooth) data were collected. Due to this reason, no paired or unpaired t-tests could be performed.

In the study by Zhang et al. (1999)[16] dentine samples with intact cementum were used for testing the preventive effect of lanthanides on carious-like lesions. Since the cementum of teeth with gingival recessions is lost due to daily tooth brushing or dental professional activities like scaling[32], a cementum-free dentine surface has been created by removing the cementum.

In order to simulate the clinical situation as close as possible, erosion and erosion prevention orientated studies[5,14,33] performed various demineralization/remineralisation protocols with erosive agent like pure acids[34] or acidic beverages[35] and remineralisation agents, such as artificial or human saliva. As the present study was planed as a primary study testing the possible preventive effect of cerium and laser irradiation against dentine erosion, no such demineralisation/remineralisation cycling was performed.

One limitation of the present study is that no intrapulpal pressure, resulting in an outwards-orientated dentine fluid flow, was simulated. This might cause an overestimation of the possible preventive effects.

In contrast to numerous other studies dealing with erosive tooth wear, the dentine wear in the present study was not determined profilometrically.[31,36,37] Due to collagen matrix exposition after erosion[38], the exact measurement of dentine wear is critical and rather technique sensitive.[39] When using optical profilometry, the remaining collagen matrix leads to an underestimation of the dentine wear as the laser beam, used for optical profilometry, cannot differentiate between demineralised organic matrix and mineralised dentine. When using contact profilometry the used stylus will penetrate the organic matrix to an unknown depth. To avoid these potential pitfalls of profilometric readings, Ganss et al. (2007)[38] suggested removing the exposed organic matrix with collagenase. Based on these methodological difficulties, the erosive dentine wear was determined by measuring the amount of calcium dissolved in the acid used for the erosive attacks in this investigation. This method has also been used in different other studies[3,27,40] and has been shown to be an adequate method for the determination of mineral loss during erosion.[41]

The used laser settings in this study were chosen according to the manufacturers' recommendations. Another study[14] concerning the possible positive effect of laser on dentine erosion used higher laser settings especially concerning the power and pulse duration. To prevent possible damages of the dentine due to too high power, we decided to stay within the setting range provided by the manufacturer.

The hypothesis of the present study that the acid resistance of the dentine, due to cerium application, could be increased can be accepted. The findings of the present study are partially in contrast to the findings by Zhang et al. (1999).[16] In the present study, the protective effect of cerium was lower compared with the protective effect of the used fluoride solution, while in the study of Zhang et al. (1999)[16] the protective effect of cerium was comparable with that of fluorides. This difference might be attributed to different concentrations of the used fluoride solution. In the present study, the concentration of the commercially available fluoride solution Elmex fluid in the present study was 10000 ppm while Zhang and co-workers (1999) only used a 500 ppm sodium fluoride solution. The higher fluoride concentration led to a better protective effect for the fluoride solution resulting in a relative lower protective effect of cerium only. The protective effect of the cerium chloride/fluoride solution combination was better than that of the cerium solution, alone which is in accordance with the findings by Zhang et al. (1999). This increased acid resistance might be attributed to the summation of the protective effect of the cerium and the fluoride.

The mechanism for the protective effect of cerium against erosion can be found in the crystal structure of hydroxyapatite and its derivative found after cerium application. Zhang et al. (1999)[16] suggested a replacement of calcium by cerium in the hydroxyapatite due to the similar atomic radius of calcium and cerium and the higher electric charge valence of cerium. This assumption was verified recently by the EDS analysis performed by Wegehaupt et al.[3] and also by the EDS analysis and EDS mapping in the present study. As the ionic radii and the electric charge valence influences the stability of apatite[42] the hydroxyapatite with cerium replacing calcium has a more stable crystal structure and reveals a greater acid resistance.

The hypothesis of the present study that additional laser irradiation could improve the acid resistance of the so treated dentine has to be rejected. Also other studies[14,22] trying to find possible protective effects of laser irradiation against dentine erosion failed to show this effect. It might be suggested that the possible mechanism (change of the chemical composition and morphology of substrates), responsible for the protective effect of laser against enamel demineralisation, does not work on dentine due to a much higher water and protein content compared with enamel, thus decreasing the contribution of the mineral phase and accentuating the role of water and protein in light absorption.[14,43] In the present study, the calcium release into the acid of the erosive attacks was higher after laser irradiation except for the placebo group. This finding might be attributed to the roughening of the surface found in the EDS mapping of the laser-irradiated samples. Due to this roughening, the sample surface is increased resulting in more minerals being dissolved by the acid.

Conclusion:

By the results of the present study it can be concluded that the combined application of fluoride and cerium chloride solutions has a significant better protective effect compared with fluoride application only. However, a simultaneous laser irradiation had no additional protective effect

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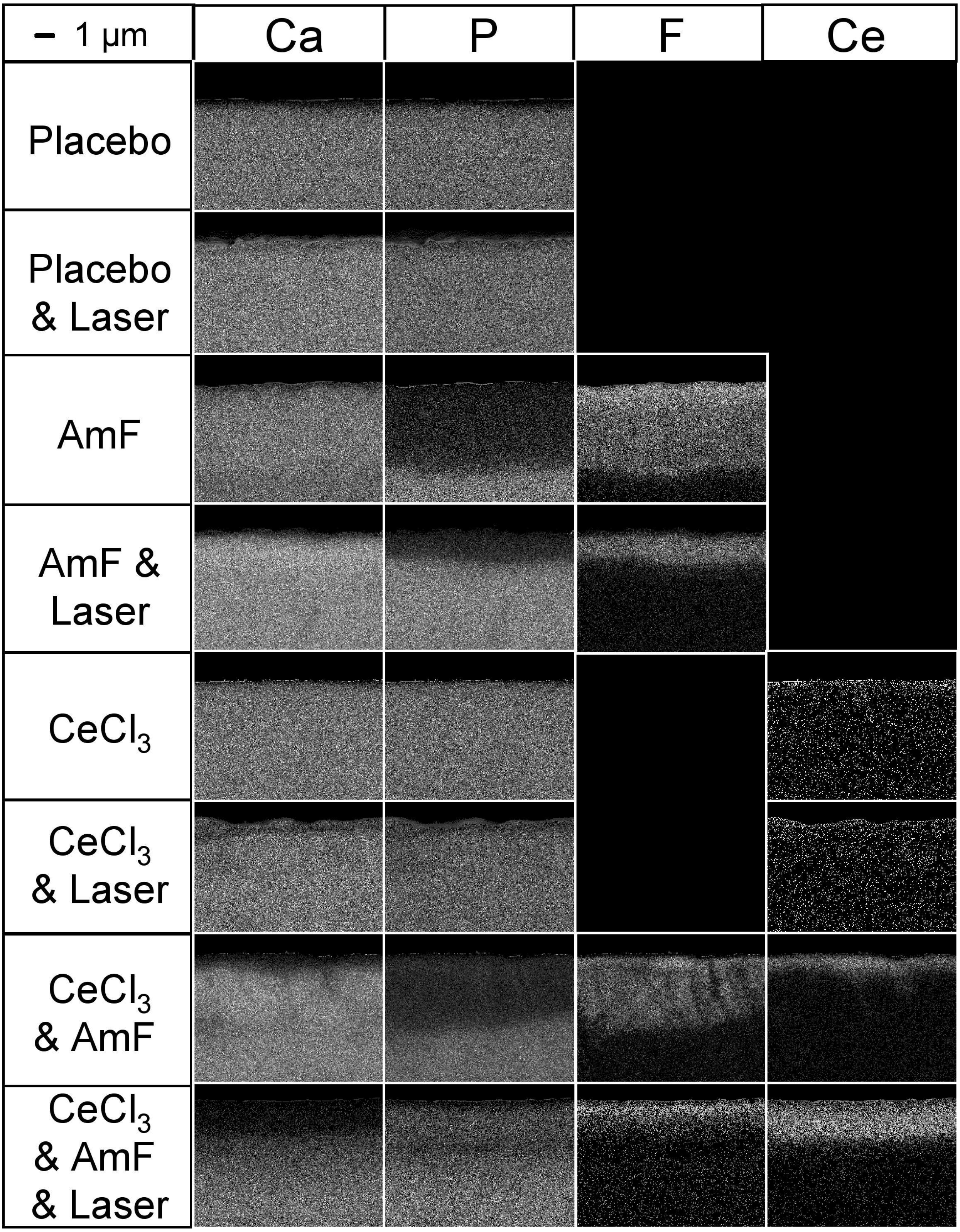
Figure captions:

Tab. 1: Mean amount (±SD) of calcium [μg] in the lactic acid of the six consecutive erosive attacks (EA 1–EA 6) and cumulative (cum) in the eight treatment groups (G1-G8).

Identical superscript capitals represent values, which are NOT statistically significantly different (read horizontally within the laser and no laser treatment group). The asterisk represents values, which are pair-wise significantly different: no laser vs. laser treatment at a respective EA.

Tab. 2: Mean (±SD) of weight percentage [wt%] of calcium, cerium, phosphor and fluoride in the dentine surface of the different groups (G1-G8) after application of the respective solutions. Comparisons are made within the elements (read vertically). Values that are not statistically significant different are marked with same lowercase letters.

Fig. 1: EDS mappings of the axial cut samples. Darker areas indicate a lower content of the respective elements.



1 µm
Ca
P
F
Ce
Placebo
Placebo & Laser
AmF
AmF & Laser
$CeCl_3$
$CeCl_3$ & Laser
$CeCl_3$ & AmF
$CeCl_3$ & AmF & Laser

Fig. 1:

	Groups							
	G1 Placebo	**G3** AmF	**G5** $CeCl_3$	**G7** AmF/ $CeCl_3$	**G2** Placebo	**G4** AmF	**G6** $CeCl_3$	**G8** AmF/ $CeCl_3$
	No laser				**Additional laser irradiation**			
EA 1	7.8 ^{A} (1.6)	0.8 ^{B*} (0.4)	3.2 ^{C*} (1.0)	0.2 ^{D*} (0.2)	7.2 ^{A} (1.7)	1.6 ^{B*} (0.4)	6.8 ^{A*} (1.7)	1.0 ^{C*} (0.4)
EA 2	7.7 ^{A} (1.1)	0.7 ^{B*} (0.4)	5.3 ^{C*} (0.9)	0.3 ^{D*} (0.5)	7.2 ^{A} (0.9)	1.3 ^{B*} (0.3)	7.2 ^{A*} (1.1)	1.0 ^{B*} (0.3)
EA 3	7.4 ^{A} (1.0)	0.8 ^{B*} (0.4)	6.1 ^{C*} (0.8)	0.2 ^{D*} (0.2)	7.3 ^{A} (1.6)	1.4 ^{B*} (0.6)	7.1 ^{A*} (1.1)	1.3 ^{B*} (0.3)
EA 4	6.8 ^{A} (0.8)	0.8 ^{B*} (0.5)	5.9 ^{A} (1.1)	0.2 ^{C*} (0.1)	7.0 ^{A} (1.6)	1.5 ^{B*} (0.7)	6.7 ^{A} (1.4)	1.5 ^{B*} (0.4)
EA 5	6.6 ^{A} (0.6)	1.0 ^{B} (0.6)	6.1 ^{A} (1.3)	0.2 ^{C*} (0.1)	6.6 ^{A} (0.6)	1.4 ^{B} (0.4)	6.8 ^{A} (1.0)	1.6 ^{B*} (0.5)
EA 6	6.3 ^{A} (0.6)	0.8 ^{B*} (0.4)	5.9 ^{A*} (0.6)	0.3 ^{C*} (0.2)	6.0 ^{A} (0.7)	1.6 ^{B*} (1.0)	6.7 ^{A*} (0.9)	1.7 ^{B*} (0.4)
cum	42.5 ^{A} (5.0)	5.1 ^{B*} (2.5)	32.6 ^{C*} (4.8)	1.5 ^{D*} (0.7)	41.3 ^{A} (5.0)	8.8 ^{B*} (2.2)	41.3 ^{A*} (6.1)	8.1 ^{B*} (1.7)

Tab. 1:

		Ca wt%	P wt%	F wt%	Ce wt%
Groups	**G1** (Placebo)	32.4 ± 3.2^{A}	16.4 ± 1.2^{A}	0.2 ± 0.2^{A}	
	G2 (Placebo & Laser)	31.8 ± 5.2^{A}	16.2 ± 1.8^{A}	0.3 ± 0.2^{A}	
	G3 (AmF)	38.6 ± 4.6^{B}	2.4 ± 0.6^{B}	35.2 ± 3.3^{B}	
	G4 (AmF & Laser)	39.6 ± 5.5^{B}	4.0 ± 0.7^{C}	34.8 ± 3.7^{B}	
	G5 ($CeCl_3$)	24.0 ± 2.8^{C}	13.8 ± 1.1^{D}	0.3 ± 0.6^{A}	11.1 ± 2.7^{A}
	G6 ($CeCl_3$ & Laser)	29.5 ± 4.3^{A}	15.7 ± 1.4^{A}	0.3 ± 0.3^{A}	6.5 ± 4.5^{B}
	G7 (AmF / $CeCl_3$)	27.0 ± 4.4^{D}	2.3 ± 0.4^{B}	30.5 ± 3.6^{C}	22.2 ± 5.1^{C}
	G8 (AmF / $CeCl_3$ & Laser)	1.9 ± 0.2^{E}	7.2 ± 0.9^{E}	12.8 ± 3.7^{D}	60.3 ± 5.7^{D}

Tab. 2: